and K104E was slightly decreased (1.3 fold and 1.4-fold increase in Kd, respectively). Fluorescence and light scattering measurements monitoring the binding of WT or mutant-myosin to pyrene-labeled actin demonstrated a very strong binding with no mutant dependent changes in Kd. These results suggest that any structural changes that may be caused by these two FHC-RLC mutations are not sufficient to affect the myosin-actin binding. Supported by NIH-HL071778 (DSC).

601-Pos Board B401
The HCM-Linked Ala13thr Mutation in the Cardiac Myosin Regulatory Light Chain Increases Isometric Force Production
Katarzyna Kazmierczak, Priya Muthu, Wenrui Huang, Ana Rojas, Michelle Jones, Yingcai Wang, **Danuta Szczesna-Cordary**.
The myosin regulatory light chain (RLC) is attached to the α-helical neck region of the myosin head, the so called lever arm, which connects the catalytic and actin binding domains with the thick filament backbone thus participating in the transmission of external forces to the myosin active site. It is understandable that mutations in the RLC associated with hypertrophic cardiomyopathy (HCM) may lead to alterations in force generation affecting cardiac muscle performance. Here, we studied the physiological consequences of an Alanine to Threonine (A13T) mutation in the N-domain of myosin RLC, found in population studies to cause HCM with a specific disease phenotype characterized by mid-ventricular obstruction. We observed an A13T-induced 30-50% increase in maximal force measured in skinned cardiac muscle fibers from transgenic Tg-A13T mice compared to control, Tg-WT and non-Tg littermates. Furthermore, a mutation-mediated 1.3-fold decrease in V_{max} and a 1.5-fold increase in K_m were observed in the actin-activated myosin ATPase activity compared with myosin from the healthy controls. The binding of Tg-myosin to pyrene-actin was similar for all groups of mice. No changes in the maximal myofibrillar ATPase or in the Ca^{2+}-sensitivity were noted. The same was true for the force-pCa relationship and the mutation did not introduce any alterations in the Ca^{2+}-sensitivity of force development. Gross morphological evaluation revealed enlarged inter-ventricular septa and left ventricles in the hearts from Tg-A13T mice, a phenotype observed in patients harboring the A13T mutation. Our results indicate that the A13T mutation may result in a hypertrophic response through abnormally increased force that may exceed the tolerance of a healthy myocardium. A decreased rate of cross-bridge turnover further demonstrates inadequate energy generation in Tg-A13T mice adding to impaired sarcomeric function. Supported by NIH-HL071778 (DSC).

602-Pos Board B402
Role of the Tail in the Regulated State of Myosin 2
HyunSuk Jung, Neil Billington, Kavitha Thirumurugan, Bridget Salzameda, Hitesh Patel, Christine R Cremo, Joseph M Chalovich, Peter D Chantler, Peter J Knight.
Myosin 2 from vertebrate smooth muscle and non-muscle sources is in equilibrium between compact, inactive monomers and thick filaments under physiological conditions, provided its regulatory light chains (RLCs) are not phosphorylated. In the inactive monomer, not only are the two heads compactly packed together, but the long tail is folded into three closely-packed segments that are associated chiefly with one of the heads. The molecular basis of the folding of the tail remains unexplained. Here, we show that compact monomers of smooth muscle myosin 2 have the same structure in both the native state and following cross-linking between Cys108 on the RLC and segment 3 of the tail. Non-specific cross-linking of the folded monomer by glutaraldehyde does not affect the compact conformation, and stabilises it against unfolding at high ionic strength. Sequence comparisons among both the RLCs and segment 3 of the tail suggest that folding of the tail is stabilised by ionic interactions between the N-terminal sequence of the RLC and the tail, and that phosphorylation of the RLC could upset these interactions. Close packing of the three tail segments may use the same ionic interactions between segments that stabilise interactions between extended tails in thick filaments. Our results support the view that interactions between the heads and the distal tail perform a critical role in reducing basal ATPase activity of myosin 2 molecules in compact monomers.

603-Pos Board B403
Two Mechanisms For Increasing Muscle Calcium Sensitivity by ROS
Sean M. Gross, Steven L. Lehman.
Cardiac muscle is sensitive to reactive oxygen species (ROS). Most measurements of calcium sensitivity following ROS exposure have shown a decrease, but others have measured no change or even an increase. One difference between studies was the activation state when ROS was applied. We therefore sought to characterize ROS-induced changes in myofilament proteins and their functional effects under different conditions of [Ca] and [ATP]. First, we identified all reactive cysteines in myofilament proteins by exposing myofibrils to a fluorescent maleimide probe under varying calcium concentrations, followed by SDS-PAGE. Only cysteines in troponin C had reactivity modulated by [Ca]. To find functional effects, we measured myofibril ATPase after exposure to 100uM DTDP in solutions at pCa 4.0 or 9.0. Ca sensitivity increased only when DTDP had been added at pCa 4.0. Next, we compared the reactivity of cysteines in myofibrils exposed to DTDP under rigor or relaxing conditions. Rigor conditions decreased reactivity of myosin cysteines but increased reactivity of actin cysteines, compared to relaxing conditions. We then measured ATPase rates in myofibrils exposed to DTDP under rigor or relaxing solutions. Exposure to DTDP in relaxing solution decreased both the maximum ATPase rate and the calcium sensitivity compared to myofibrils not exposed to DTDP (control). In contrast, when DTDP was exposed in rigor solution the minimum ATPase rate was increased from control, and there was an increase in calcium sensitivity. In conclusion, we found two activation dependent mechanisms to increase calcium sensitivity: the first calcium-dependent and specific to troponin C, the second dependent on whether myosin was bound to actin.

604-Pos Board B404
Cardiac Sarcomeric Proteins Reveal Differential Susceptibilities to Oxidative-Stress
Laura Harvey, Amelia Sumandea, Gail Sievert, **Marius P. Sumandea**.
Many models of oxidative stress lead to heart failure syndromes that are not associated with changes in Ca^{2+}-homeostasis, and are likely attributable to oxidative stress-dependent modifications of sarcomeric proteins. Yet, the possible sarcomeric targets and specific modifications are poorly understood. In the present study, we evaluate whether cardiac sarcomeric proteins manifest different sensitivities to metal (iron) catalyzed oxidative stress. Exposure of rat myocytes to H_2O_2 lead to the production of: i) myofilament-protein aggregates resistant to reducing (DTT) and denaturing (urea/thiourea) conditions; and ii) myofilament breakdown, even in the presence of cell permeable protease inhibitors. Pre-incubation of myocytes with cell-permeable metal chelators (like desferoxamine) completely abolished myofilament breakdown and aggregation. Similar myofilament-protein aggregates were detected in failing rat and human myocardium. Isolated rat ventricular myofibrils exposed to H_2O_2 and iron (Fe^{2+}) closely recapitulate cellular results. Dose dependent experiments reveal that most sarcomeric phosphoproteins undergo dephosphorylation and breakdown at lower oxidative stress levels compared with non-phosphoproteins.

605-Pos Board B405
Expression of Slow Tropomyosin (TM-) in Fast Fibers of Extraocular Muscles
Peter J. Reiser, Sabahttin Bicer.
We previously reported marked differences in the myosin heavy and light chain (MHC and MLC) isoform composition of fast and slow fibers between the global and orbital layers of domestic dog extraocular muscles (EOM) (Bicer & Reiser, Invest. Ophthalmol. Vis. Sci. 45:138-143, 2004 and 50:157-167, 2009). In addition, many dog extraocular fibers, especially from the muscle orbital layer, have MHC and MLC isoform patterns that are distinct from those in limb skeletal muscles. The results of these studies also suggested possible differences in the tropomyosin (TM) isoform composition of fast fibers between the two EOM layers, based upon gel electrophoretic mobility patterns. The objective of this study was to determine whether differences in TM isoform expression exist between fast global and fast orbital fibers in dog EOMs. TM isoforms in global and orbital single fibers were identified by SDS-PAGE and immunoblotting. Fast and slow fibers in the global layer express fast TM-α and slow TM-γ, respectively, plus TM-β, identical to fast and slow fibers in limb muscles. Slow orbital fibers express TM-β and TM-γ, as expected. Fast orbital fibers, on the other hand, have an unusual pattern of TM-γ, along with TM-β and TM-α. The same TM isoform expression patterns were found in Sprague Dawley rat EOM fibers. Co-expression of all three TM isoforms was not observed in single fibers in limb muscles of either species. These results contribute to the understanding of the elaborate diversity in contractile protein isoform expression among mammalian EOM fibers. Supported by the National Science Foundation.

606-Pos Board B406
Actin Binding Sites of Tropomyosin: An Evolutionary Structural Bioinformatics Analysis
Bipasha Barua, Sarah E. Hitchcock-DeGregori.
Tropomyosin (Tm) is a two-chained, α-helical coiled coil protein that associates end-to-end to form a continuous strand along actin filaments and regulates the functions and stability of actin. Mutations in Tm cause skeletal and cardiac muscle myopathies. We carried out a phylogenetic analysis of tropomyosin to